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PRIMER NOTE

Characterization of eight polymorphic microsatellite markers in the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois)

O. P. PERERA,* G. L. SNODGRASS,* B. E. SCHEFFLER,† J. GORE* and C. A. ABEL*
* *Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA* †*Genomics Core Facility, Cat Fish Genetics Research Unit, USDA-ARS, Stoneville, MS 38776, USA*

Abstract

A partial genomic library of the tarnished plant bug, *Lygus lineolaris*, enriched for microsatellite sequences was screened to identify marker loci. Eight polymorphic loci suitable for population genetic studies were identified by screening 192 field-collected insects. The observed number of alleles ranged from four to 21 with an average of 12.25 (SE ± 1.94) while the effective number of alleles ranged from 1.23 to 11.05 with an average of 4.49 (SE ± 1.15). No linkage disequilibria or significant deviations from Hardy–Weinberg expectations were detected at any of the loci. Seven of the eight *L. lineolaris* microsatellite loci were transferable to *Lygus hesperus*.

Keywords: genetic markers, *Lygus*, microsatellite, SSR, tarnished plant bug

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The tarnished plant bug, *Lygus lineolaris*, is found in all agricultural regions of the United States and Canada and has been reported to feed on 328 different plant species (Young 1986). Tarnished plant bugs are controlled in cotton exclusively with insecticides and in the mid-South have developed varying levels of resistance to several classes of insecticides (Snodgrass 1996). In order to study population genetic characteristics of the tarnished plant bug, we set out to develop a comprehensive set of genetic markers for this species.

A partial genomic library of *L. lineolaris* enriched for di-, tri-, and tetra-nucleotide microsatellite sequences was constructed following the enrichment protocol of Hamilton *et al.* (1999) with a few modifications. Modifications included the use of three pools of biotinylated oligonucleotides and magnetic beads from a PolyA+ Tract mRNA isolation system (Promega) for enrichments and cloning of polymerase chain reaction (PCR) products directly into pCR 2.1 TOPO vector (Invitrogen). Synthetic biotinylated oligonucleotides (Integrated DNA Technologies, Inc.) containing 10 different microsatellite repeat sequences were divided into three groups based on the hybridization temperature and the absence of complementary nucleotide sequences. The hybridization temperature of the group 1 oligonucleotides [$(AC)_{13}$ and $(AGC)_6$] was 58 °C and oligonucleotides in group 2 [$(AAC)_6$, $(AAG)_8$, $(ACT)_{12}$, $(ATC)_8$, $(AG)_{14}$] and group 3 [$(AAAC)_6$, $(AAAG)_6$, $(AAT)_6$] had hybridization temperatures of 52 °C and 48 °C, respectively. Master mixes containing 2 μM of each oligonucleotide were prepared and 2 μL of each master mix was combined with 7 μL of SNX linker-ligated DNA, 50 μL of 2× hybridization buffer (12× SSC; 0.1% SDS), and adjusted to 100 μL with 41 μL of water. Reaction mixes were denatured, annealed at a temperature appropriate for each group, and enriched by capturing with streptavidin-coated magnetic beads (Hamilton *et al.* 1999). Enriched sequences were PCR amplified using the SNX-F primer and subjected to a second round of enrichment followed by PCR amplification with the SNX-F primer. Resulting PCR products were cloned into the pCR 2.1 TOPO cloning vector and 2 μL of the ligation mix was used to transform One-Shot Mach1-T1R competent cells (Invitrogen). Recombinant clones were selected on Luria Bertani agar plates containing 50 μg/mL kanamycin and 40 μg/mL X-Gal. Nucleotide sequencing was carried out at the USDA-ARS Mid-South Area Genome Research Center, Stoneville, Mississippi.

Correspondence: O. P. Perera, Fax: 662-686-5421; E-mail: operera@msa-stoneville.ars.usda.gov

Table 1 Characteristics of eight microsatellite loci isolated in *Lygus lineolaris* collected from Washington County, Mississippi

Locus (accession no.)	Primer sequence (5′–3′)	Cloned allele		Observed allele size range (bp)	Sample size	n_o	n_e	H_O, H_E
		Repeat sequence	Size					
LlMS1-15 (EF112178)	F: AAATGAAAGCGAAGAGACGAGTTG R: GCAACATCCCGCTGTAACGTA	$(CA)_8$	109	88–128	179	4	1.23	0.2011, 0.1914
LlMS1-26 ‡ (EF112179)	F: TGTGCCAAAGCAAAGACATTAAAA R: GTACTCTGCGGAGTGACATGAAAA	$(CA)_7$	128	109–145	188	9	1.31	0.2181, 0.2374
LlMS1-43 ‡ (EF112180)	F: TGAGAGTGAATGTTCAACGTCAG R: CTGAATCTTGTCTTGGGTAGATCG	$(GT)_9$	120	105–151	184	21	11.05	0.8152, 0.9120
LlMS2-25 ‡ (EF112181)	F: TCCTCAGCAAACACCATGAATAGA R: CAGTGCTCCAGGACATTCAAGTTA	$(AGT)_4$	152	114–190	187	19	2.6	0.5882, 0.6184
LlMS2-47 ‡ (EF112182)	F: GAACACATCCTGTTGTGCTCCTTA R: TTTCAAACCCCACTTTGAAACCT	$(AGA)_6$	118	106–132	185	10	5.23	0.6162, 0.8112
LlMS3-16 ‡ (EF112183)	F: TATTACCTTCTTCAGGGCTCGAAA R: TCGCATGGTGTTTTTCAAAGTCTA	$(GAT)_7$	154	143–165	186	13	3.62	0.5914, 0.7261
LlMS3-60 ‡ (EF112184)	F: GTCTTGAAAACAGACAATCCGCTT R: TGGCTAAATCCCGACAATATTCAT	$(GAT)_6$	97	86–108	186	12	6.78	0.7473, 0.8550
LlMS3-91 ‡ (EF112177)	F: TGACGATTGTTATGTACCATTGCTG R: CTTATTGGTGCCTAATCCGAACTG	$(GAT)_6$	145	139–164	181	10	4.09	0.6077, 0.7577
				Mean	184.5	12.25	4.49	0.548, 0.639
				S.E.	1.09	1.94	1.15	0.079, 0.098

Locus names, GenBank Accession nos, forward (F) and reverse (R) primer sequences, repeat sequence and length of the cloned allele, size range of observed alleles, observed and effective allele numbers (n_o and n_e, respectively), and observed and expected heterozygosities (H_O and H_E, respectively) for each locus is given. Sample size indicates the number of insects (out of 192) scored for each locus.
‡Loci transferable to *Lygus hesperus*.

Microsatellite detection and primer design was performed using SSRFINDER software (Sharopova *et al*. 2002). The design conditions were set to obtain primer pairs with a difference of less than 1 °C in the T_m, within the T_m range of 60–65 °C (target 63 °C). The length for primers was 20–24 nucleotides (target 24 nucleotides). In order to use a universal fluorescent-labelled primer in genotyping reactions, a 'tail sequence' (5′-CAGTTTTCCCAGTCACGAC-3′) identical to the universal fluorescent-labelled primer was added to each of the forward primers and a stabilizer sequence (5′-GTTT-3′) was added to each of the reverse primers (Taliercio *et al*. 2006). Amplification reactions were set up with 1 μL of approximately 20 ng/μL genomic DNA, 0.8 μL of a primer mix [0.4 pM forward primer, 1.2 pM reverse primer, 1.2 pM 6-carboxyfluorescein (6-FAM) labelled universal primer], 0.1 μL of Titanium *Taq* polymerase (BD Biosciences), 1 μL of 10 mM dNTP mix, and 0.5 μL of 10× Titanium *Taq* polymerase buffer in a 5-μL volume. Cycling conditions were: initial denaturation for 2 min at 95 °C followed by 1 min at 60 °C and 30 cycles of 15 s at 95 °C, 15 s at 60 °C, and 30 s at 72 °C. The PCR products were run as 1/9 dilutions on an ABI 3700XL genetic analyser with ROX-labelled markers. Peaks were scored with GENEMAPPER (Applied Biosystems) software and confirmed manually. Statistical analysis was carried out using GENEPOP web version 3.4 (Raymond & Rousset 1995) software.

Eight primer pairs suitable for population genetic analyses were identified from a set of 33 primer pairs. Characteristics of these eight microsatellite loci in *L. lineolaris* collected from a location in Washington County, Mississippi (N = 192) are given in Table 1. The observed and effective allele numbers ranged from four to 20 and 1.23–11.05, respectively. The observed and effective number of alleles per locus averaged 12.25 (SE ± 1.94) and 4.49 (S.E. ± 1.15), respectively. Fisher exact tests using Markov Chain method (1000 dememorization steps, 100 batches, and 1000 iterations per batch) did not indicate deviations from Hardy–Weinberg equilibrium at any of the loci or linkage disequilibrium between any pair of loci. A pair of loci was considered in LD if the P value associated with test was ≤ 0.01. In addition, genotyping tests with *Lygus hesperus* DNA (from Lubbock, Texas, N = 12) revealed that all loci, with the exception of LlMS1-15 locus are transferable to *L. hesperus*.

In the present study, we characterized eight polymorphic microsatellite markers suitable for population genetic studies in *L. lineolaris*. Seven of these markers were also suitable for genotyping *L. hesperus*. The selection of primer pairs with an optimal T_m of 60 °C facilitates high throughput genotyping of samples under uniform PCR conditions. Extensive spatial and temporal sampling of *L. lineolaris* populations have been carried out and analysis of these populations are in progress.

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